

THE TAXONOMY AND CHEMISTRY  
OF PINUS ESTEVEZII

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IN HIS ORIGINAL DESCRIPTION of *Pinus pseudostrobus* var. *estevezii* Martínez, Martínez (1945, p. 188) pointed out that "this pine, from its general aspect, resembles *P. pseudostrobus* Lindl. and *P. montezumae* Lamb. but presents some characteristics, principally in the cone, that in my opinion merit its consideration as a variety of the first" (author's translation). Loock (1950) generally followed Martínez's description of *P. pseudostrobus* var. *estevezii* and also noted (p. 156) that the species is "related to both *P. montezumae* and *P. pseudostrobus*, but more closely to the latter in that the bark of the young trees and upper part of the stems is smooth." Zobel and Cech (1957) collected *P. pseudostrobus* var. *estevezii* in the state of Nuevo León and noted the difficulty they experienced in classifying the five-needled hard pines, primarily because of the many intermediate or intergrading forms. Gausson (1960) elevated *P. pseudostrobus* var. *estevezii* to specific status, but the change in rank is invalid since he did not refer to the original publication. Mirov (1961) followed Martínez and Loock in his treatment of *P. pseudostrobus* var. *estevezii*. Shaw (1909, 1914), Standley (1929), Miranda and Hernández (1963), Rojas-Mendoza (1965), Critchfield and Little (1966), and J. Rzedowski (1978) made no mention of the variety.

In this paper both morphological and biochemical characteristics are used as a basis for elevating *Pinus pseudostrobus* var. *estevezii* to specific rank. There is now considerable precedence for such an approach. Mirov (1948, 1958, 1961), Zobel (1951), Williams and Bannister (1962), Smith (1967), and Rockwood (1973) used chemical composition of turpentine as an important diagnostic character in their studies of different species of pines, their hybrids, and their classification. Evidence (discussed below) that I have collected from numerous field observations, morphological studies, and chemical analyses of stem oleoresins from *Pinus montezumae*, *P. pseudostrobus*, and *P. pseudostrobus* var. *estevezii* strongly indicates that var. *estevezii* should be considered as a distinct species.

***Pinus estevezii* (Martínez) Perry, comb. et stat. nov.**

FIGURE 1.

*Pinus pseudostrobus* var. *estevezi* Martínez, Las Pinaceas Mexicanas I: 188-192. figs. 158-160. 1945. TYPE: Mexico, Nuevo León, Santa Catarina, Cañon de las Mieleras, June, 1941, Martínez 3433 (holotype, MEXU).

Tree 15-20 m tall, d.b.h. .75-1 m; branches large, often rather low on



FIGURE 1. *Pinus estevezii* growing near town of Iturbide, Nuevo León, Mexico.

stem, horizontal to slightly ascending, crown often dense and rounded. Bark on mature trees reddish brown, thick, divided into longitudinal plates by deep vertical and horizontal fissures. In young trees upper part of stem quite smooth since bases of needle bracts not decurrent and soon merging into bark. Leaves in fascicles of 5 (rarely less), bright green, 20–30(–35) cm long, ca. 1 mm thick, stiff, edges finely serrate; stomata present on all 3 faces; hypoderm irregular, 2 to 4 layers of cells with many shallow penetrations into chlorenchyma; resin canals 3 to 5, medial; endodermis with thickened outer cell walls; vascular bundles 2, quite distinct; fascicle sheath ca. 20 mm long, persistent, pale brown, not gummy or resinous. Conelets erect, reddish purple, subterminal, solitary or borne 2 to 4 together; scales thick, bearing strong, up-curved prickle. Cones yellowish brown to brown, 10–13 by 7–8 cm, long-ovoid or conoid, asymmetric, often reflexed (occasionally erect and symmetrical); opening at maturity, generally in December and January; borne on thick, strong peduncles ca. 1 cm long that remain attached to branchlet along with few basal cone scales when cone falls. Scales hard, strong, 25–30 by 12–15 mm, apex slightly rounded to pointed, apophysis subpyramidal, with prominent transverse keel, the umbo raised, prominent, grayish, armed with strong, persistent prickle most often curved upward

(FIGURE 2). Seeds dark brown, ovoid to long-ovoid, 6–7 mm long; wing detachable, brown, ca. 25 by 7–9 mm.

#### TYPIFICATION

In his original description of *Pinus pseudostrabus* var. *estevezii*, Martínez (1945, p. 189) stated "habitat in Cañon de las Mieleras, Santa Catarina, Nuevo León, Typus in Herb. Instituto de Biología, Mexico"; he did not specify a date or a collection number. His illustrations were apparently taken from the type specimen. He also cited (p. 191) collections from "Sierra de Santa Catarina, N.L. en los Picachos de Sabinas, N.L. y en Los Lirios, Arteaga, Coah."; collectors' names and numbers were again not given. While in Mexico City in 1979, I found a specimen at the Herbario Nacional del Instituto de Biología, Universidad Nacional Autónoma de México, bearing a glued label that reads (handwritten) "*Pinus pseudostrabus* var. *Estevezii* Martínez; Cañon de Mieleras, Sta. Catarina, N.L. var. nova, tipo, Junio 1941, Num: 3433" and (printed) "Prof. Maximino Martínez." This specimen, consisting of a single branchlet bearing needles and two mature, open cones, can reasonably be considered the holotype of *Pinus pseudostrabus* var. *estevezii* Martínez.

#### DISTRIBUTION

I have collected and observed *Pinus estevezii* in the type locality. In Nuevo León I have also seen it northeast of Galeana, near the village of Dieziocho



FIGURE 2. *Pinus estevezii*: cones, conelets, foliage, and branchlets.

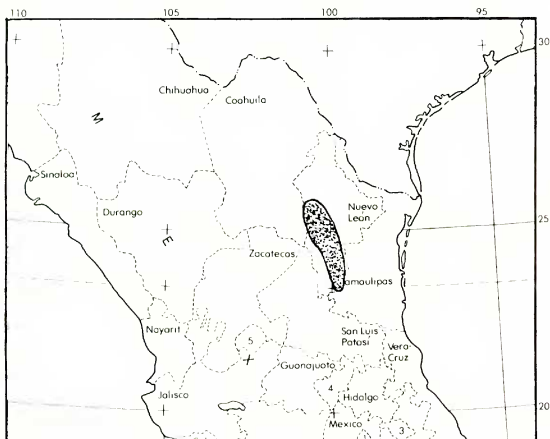
de Marzo, at the foot of Cerro Potosí in association with *P. cembroides* Zucc., *P. arizonica* Engelm. ex Rothr. var. *stormiae* Martínez, and south of Zaragoza. In 1978 I collected it in the state of Tamaulipas at 1800 m alt. near the dirt road from Palmillas to Miquihuana. This was a small, scattered stand of trees growing in association with *P. cembroides* and *Quercus* spp. At all of these locations the site was generally dry, rocky, and overgrazed.

Look (1950) and Mirov (1967) reported *Pinus estevezii* occurring in the states of Coahuila and Nuevo León. Zobel and Cech (1957) collected *P. estevezii* west of Iturbide, Nuevo León, and Mittak (pers. comm.) reported a collection (*Mittak 8921*) from 47 km west of Linares, Nuevo León, at 1420 m alt. Other records of the species are as follows: Mexico, Nuevo León: Dulces Nombres, and E to border into Tamaulipas, 1800 m, *F. G. Meyer & D. J. Rogers 3087* (GH); Sierra Madre above Monterrey, 770–1220 m, *Pringle 10170* (GH); Sierra Madre, Monterrey, *C. H. Mueller & C. T. Mueller 33* (A).

Thus the range of *Pinus estevezii* extends from near the city of Monterrey, south along the Sierra Madre Oriental, into the southeastern corner of the state of Tamaulipas near the town of Miquihuana (MAP 1).

#### DISTINCTION BETWEEN *PINUS ESTEVEZII*, *P. MONTEZUMAE*, AND *P. PSEUDOSTROBUS*

Martínez (1945) and Look (1950) agreed regarding differences between the cones, stems, and branchlets of *Pinus pseudostrobus* and its variety *es-*



MAP 1. Distribution of *Pinus estevezii* in Mexico.

*tevezii* (see Martínez, 1945, pp. 190, 191, *figs. 159, 160*; Loock, 1950, pp. 156, 157, *pl. A*). Loock (1950) showed the differences between branchlets of *P. montezumae* and *P. pseudostrobus*, and between the cones and cone scales of *P. pseudostrobus* and its varieties.

Regarding differences between *Pinus pseudostrobus* var. *estevezii* and *P. montezumae*, both Loock and Martínez stressed that in *P. montezumae* bases of the leaf bracts are decurrent, leaving the branchlets and stems of young trees very rough and scaly, while in *P. pseudostrobus* var. *estevezii* (hereafter in this paper referred to as *P. estevezii*) the leaf bract bases on stems of young trees are not decurrent and soon merge into the bark, leaving the stems smooth (FIGURE 3). On the older branchlets of *P. estevezii*, leaf bract bases are raised and prominent, but they are neither scaly nor as rough as in *P. montezumae*.

There are other differences between the three taxa. Mature trees of *Pinus estevezii* rarely attain a height of 20 m, and the crowns are generally low and broad, often with the lower branches extending almost to the ground. In sharp contrast, both *P. pseudostrobus* and *P. montezumae* attain heights of 30–40 m and have characteristically long, clear boles and rounded crowns. In addition, bark of *P. estevezii* is reddish brown and furrowed by deep fissures into long, rectangular plates; that of mature *P. montezumae* and *P. pseudostrobus* is dark grayish brown and has smaller plates.

Differences between the cones of *Pinus pseudostrobus* and *P. estevezii* are generally quite consistent. Cones of *P. pseudostrobus* range in length from 8 to 10 cm, while those of *P. estevezii* are 10–13 cm. Cone scales of *P. pseudostrobus* are not as stiff and strong as those of *P. estevezii*. Apophyses

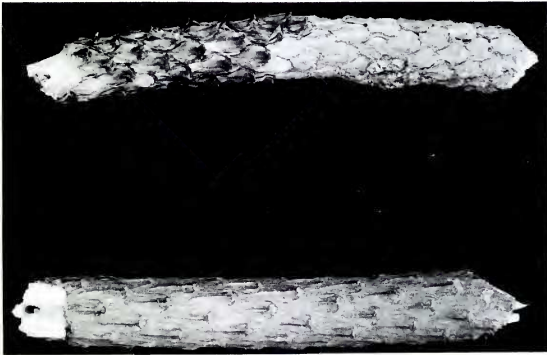


FIGURE 3. Above. *Pinus montezumae*, decurrent leaf bract bases on stem of young plant. Below: *P. estevezii*, nondecurrent leaf bract bases. Both  $\times 0.9$ .

TABLE 1. Summary of differences between *Pinus estevezii*, *P. pseudostrobus*, and *P. montezumae*.

CHARACTER	SPECIES		
	<i>P. estevezii</i>	<i>P. pseudostrobus</i>	<i>P. montezumae</i>
CONES	10-13 by 7-8 cm, long-ovoid or conoid	8-10 by 5-7 cm, generally ovoid	13-15 by 7-10 cm, ovoid or conoid
CONE SCALES	Hard, strong, thick; apophyses raised to subpyramidal, with prominent transverse keel; umbo raised, armed with strong, persistent prickle, generally curved upward toward apex	Neither as hard nor as thick as in <i>P. estevezii</i> ; apophyses slightly keeled; umbo small, occasionally depressed, armed with small, deciduous prickle	Scales hard, strong, thick; apophyses raised, subpyramidal, with strong transverse keel; umbo slightly raised, armed with small, deciduous prickle
LEAVES			
DIMENSIONS	20-30(-35) cm long, 1 mm thick	20-25(-30) cm long, ca. 0.7 mm thick	15-25(-30) cm long, 0.7-1 mm thick
HABIT	Stiff, erect	Slender, flexible, generally drooping	Variable from slender and flexible to stiff and erect

INTERNAL STRUCTURE	Hypoderm irregular, with many shallow penetrations into chlorenchyma; resin canals 3 (to 5), medial	Hypoderm uniform to irregular, with few shallow penetrations into chlorenchyma; resin canals 2 or 3 (or 4), medial	Hypoderm uniform to irregular, with few slight penetrations into chlorenchyma; resin canals 3 to 5 (or 6), medial
STEM OF YOUNG TREE	Smooth; bases of leaf bracts not decurrent, merging into bark, leaving stem smooth	Smooth; bases of leaf bracts not decurrent, merging into bark, leaving stem smooth	Rough; bases of leaf bracts decurrent, leaving stem rough and scaly
FORM OF MATURE TREE	15–20 m tall; often with large, low branches; crown dense, broadly rounded	30–40 m tall; stem clear; crown narrow, rounded	30–40 m tall; stem clear; crown narrow, rounded
TURPENTINE	Usually high amounts of heptane and small amounts of nonane; often with high $\alpha$ -pinene; sometimes with high myrcene, limonene, methyl chavicol, and $\beta$ -phellandrene	Usually with very high amounts of $\alpha$ -pinene, rarely with high myrcene; heptane, octane, and nonane usually absent	Usually with very high amounts of $\alpha$ -pinene, rarely with high myrcene; heptane, octane, and nonane usually absent
ALTITUDINAL RANGE (m)	800–1800	1600–3200	1700–3400

of *P. estevezii* cones are strongly keeled, while those of *P. pseudostrobus* are not. The prickle of *P. pseudostrobus* scales is small, weak, and early deciduous; that of *P. estevezii* is prominent, curved upward, and persistent.

The cones of *Pinus montezumae* are slightly longer than those of *P. estevezii* (13–15 cm vs. 10–13 cm). Cone scales in both species are hard, strong, and thick, with apophyses raised to subpyramidal. However, the umbo is armed with a small, deciduous prickle in *P. montezumae*, but with a strong, persistent prickle that is generally curved upward in *P. estevezii*.

Differences in altitudinal range are also quite marked. *Pinus estevezii* is generally found growing at 800–1500 m alt., while *P. pseudostrobus* usually occurs at 1600–3200 m, and *P. montezumae* at 1700–3400 m. TABLE 1 summarizes the differences between the three species.

#### RELATIONSHIPS OF PINUS ESTEVEZII

Both Martínez (1945) and Loock (1950) postulated a close relationship between *Pinus pseudostrobus*, *P. estevezii*, and *P. montezumae*. Martínez (1945) showed the relationships between the Montezuma, Ponderosa, and Pseudostrobus pine groups. His fig. 169 shows *P. pseudostrobus* var. *estevezii* and *P. pseudostrobus* var. *coatepecensis* Martínez forming a connecting link between the *P. pseudostrobus* and *P. montezumae* groups. Mirov (1967) referred to three great pine complexes of America: A, the *P. ponderosa* Lawson complex; B, the *P. montezumae* complex; and C, the *P. pseudostrobus* complex. His fig. 9-4, almost identical to Martínez's fig. 169, also showed *P. pseudostrobus* var. *estevezii* and *P. pseudostrobus* var. *coatepecensis* forming connecting links between the *P. pseudostrobus* and *P. montezumae* groups.

In an effort to clarify these relationships, I collected samples of xylem oleoresin from trees of *Pinus pseudostrobus*, *P. montezumae*, and *P. estevezii*. These have now been analyzed, and the results are shown in TABLE 2. Information regarding collection and analysis of the oleoresins is given in the APPENDIX.

It can be seen that turpentine of *Pinus estevezii* has an entirely different composition than that of *P. pseudostrobus* and *P. montezumae* (TABLE 2). Turpentine of *P. pseudostrobus* and *P. montezumae* is almost entirely  $\alpha$ -pinene. In both species there were a few trees with a large proportion of myrcene. In sharp contrast, *P. estevezii* consistently had high heptane and a smaller but very consistent percentage of nonane. These light hydrocarbons were found in neither *P. pseudostrobus* nor *P. montezumae*. In addition, there were trees of *P. estevezii* with significant amounts of five other compounds:  $\alpha$ -pinene (61% of the trees had high amounts); myrcene (30%); limonene (8%);  $\beta$ -phellandrene (15%); and methyl chavicol (15%).

These are the first published data on analyses of turpentine from *Pinus estevezii*. However, Iriarte (1946) analyzed the turpentine of *P. pseudostrobus* from Mexico and found it to consist almost entirely (over 90%) of d- $\alpha$ -pinene, and this was confirmed by Brummer's (1978) analysis of turpentine from Guatemalan specimens. Both investigators reported a complete absence of the light hydrocarbons (heptane, octane, and nonane).



TABLE 2. Turpentine composition\* of xylem oleoresin from *Pinus estevezii*, *P. pseudostrobus*, and *P. montezumae*.

Species	Sample number	Heptane	Octane	Nonane	$\alpha$ -Pinene	Camphene	$\beta$ -Pinene	$\Delta^3$ -Carene	Myrcene	$\alpha$ -Terpinene	Limonene	$\beta$ -Phellandrene	$\beta$ -Cymene	Terpinolene	$\alpha$ -Fenchol	Terpinene- $\alpha$ ol	$\beta$ -Caryophyllene	Methyl chavicol	$\alpha$ -Terpineol	
<i>P. estevezii</i>	H1	69	12	5	4	2	1	1												
	H2	36	18	14			TR <sup>§</sup>	1	26											6
	H3	24	6	8	4			2	1	23										3
	H4	30	12	13	5			3	1	13		26		3						2
	H5	15	6	7	22	3	4	3	35			15		4						4
	H6	49	18	15			5		3			7								3
	H7	33	10	5	4	TR			1		36									2
	H8	46	9	25	5	4	2		2	1	1									TR
	H9	42	9	6	11	4	6	2	3			2								10
	H10	40		11	28	4	4	TR	3			1	1							7
	H11	27		6	53	6			2			TR	1							13
	H12	49		16	18	4	3													8
	H14	28		11	38	5	7		5			1								8
	% High <sup>1</sup>		100	0	55	61	0	0	0	30	8	15		0				0	15	
<i>P. pseudo-strobus</i>	1			58	1	2	2	31				TR							1	
	2			81	1	TR	1	12		TR		TR		1					1	
	3			83	1	5	TR	4		4				TR					TR	
	4			57	1	3	2	29		TR				1					TR	
	5			91	1	TR	1	2		1									2	
	6			94	1	TR	1	3		1									2	
	7			92	1	3	1	3		1									1	
	8			68	1	3	2	22		1		TR	1						TR	
	9			87	3	3	1	3		1									1	
	10			89	1	3	1	3		1		TR				1			1	
% High <sup>1</sup>		100	0	0	0	0	40	0	0	0	0	0	0	0	0	0	0	0	0	
<i>P. montezumae</i>	M150			86	6	2	3				2									
	M152			96	2	1														
	M153			92	3	2	1			1		TR							1	
	M154			89	5	2	1			1		TR							2	
	M155			70	2	1		19		6										
	M158			98	2															
	M160			92	4	2	1			1		TR							1	
	M161			91	4	2	1			1		TR							TR	
	M162			89	3	2	1			5		TR							TR	
M163			93	1	5															
% High <sup>1</sup>		100	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	

\*Percent of total turpentine.

<sup>1</sup>Percent of trees having relatively high amounts.<sup>§</sup>TR = trace.

Both Iriarte (1946) and Mirov (1951) reported that turpentine collected from *Pinus montezumae* from the state of Michoacán, Mexico, was 96–98% d- $\alpha$ -pinene. Neither author reported the presence of any light hydrocarbons.

More recently, with the development of gas chromatography, studies of the chemical composition of pine turpentine have greatly expanded. Squillace (1976) provided an outstanding summary of investigations in this field and noted (p. 120) that "genetic studies have shown that monoterpene composition varies greatly among trees within species and is strongly inherited." He also pointed out (p. 121) that "high versus low levels of some monoterpenes are controlled by single genes. Such traits are very useful as gene markers for identification of hybrids and relatives and for studies of the degree of natural inbreeding and of population structure."

As noted earlier in this paper, Martínez (1945), Loock (1950), and Mirov (1967) stated that *Pinus estevezii* is closely related to *P. pseudostrobus* and *P. montezumae*. However, the distinctive chemical differences between its turpentine and that of *P. montezumae* and *P. pseudostrobus* clearly rule out the possibility of a hybrid origin of *P. estevezii* involving *P. montezumae* or *P. pseudostrobus* as the parental species.

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#### APPENDIX. Collection and analysis of oleoresins.

##### COLLECTION

Samples of xylem oleoresin were collected from 14 trees of *Pinus estevezii* growing near the highway from Linares to Galeana, 2 km west of Iturbide, Nuevo León, Mexico. This was an open, rather scattered stand of pines at 1650 m alt. on gravelly-rocky, heavily grazed slope. No other species of pines was associated with this group. The trees sampled were 35-70 cm d.b.h. and 15-20 m high. A hole ca. 0.5 cm in diameter was drilled into the stem of each tree about 75 cm from the ground (October 25, 1978), and a threaded glass vial was immediately screwed tightly into the hole. The next day the vials were collected and each one was covered with a threaded, gasketed cap. Resin flow from all trees was abundant and uniformly pale amber in color. Specimen number *J. P. Perry MEX 78-1043* was collected as a composite voucher for these trees and has been deposited in the herbarium at nsc.

Samples of xylem oleoresin were collected from *Pinus pseudostrobus* trees by Biol. Xavier Madrigal Sanchez at Puenteillas near the village of Dos Aguas, Coahuila, Michoacán, Mexico. The trees were growing on a gravelly clay slope at 2370 m alt. Associated species were *Pinus michoacana* var. *cornuta* Martínez, *Pinus douglasiana* Martínez, and *Quercus* spp. The sample trees were 30-54 cm in diameter and 25-30 m high. The sampling procedure was the same as for *P. estevezii*. Vials were placed on ten trees on February 8, 1979, and were collected on February 12. Resin flow from all trees was abundant and uniformly clear white in color. *X. Madrigal Sanchez CIFO 3226* (deposited at the herbarium of the Centro de Investigación Forestal Oeste, Uruapan, Michoacán, Mexico) was collected as a voucher for the trees.

Samples of xylem oleoresin were collected from ten *Pinus montezumae* trees growing near the Mexico City-Toluca highway, adjacent to the federal park, Desierto de Los Leones, México, D. F. This was a fully stocked stand of mature trees growing at 2750 m alt. in a gently sloping area of deep volcanic soil. Associated tree species were *P. patula* Schlecht. & Cham., *Abies religiosa* (H.B.K.) Schlecht. & Cham., and *Quercus* spp. The ten sample trees were 40-60 cm in diameter and 25-35 m high. Oleoresin samples were obtained in the same manner as those taken from *P.*

*estevezii* and *P. pseudostrobis*. Vials were placed on the trees on January 27, 1980, and collected on January 29. Resin flow from all trees was abundant and uniformly clear white in color. *J. P. Perry MEX-150* was collected as a composite voucher for these trees and has been deposited in the herbarium at nsc.

#### ANALYSIS

Most of the analyses were performed by a single chemical consulting laboratory; however, a few samples were analyzed by another laboratory. The following gas chromatographic conditions and equipment were used for analysis of most of the pine resin samples.

Turpentine from each sample was separated from the resin and extraneous matter by steam distillation (kept alkaline to prevent acid isomerization).

The chromatograph used was a Varian Series 1700 with a thermal conductivity detector. A stainless steel column  $10' \times \frac{1}{8}"$  diameter packed with 15% carbowax 20M on "chromosorb W" was injected with  $1.5 \mu\text{l}$  of sample. Injector temperature was  $210^{\circ}\text{C}$ , detector temperature  $225^{\circ}\text{C}$ , and the column oven programmed from  $75^{\circ}$  to  $220^{\circ}\text{C}$  with a  $4^{\circ}\text{C}/\text{minute}$  temperature rise. The carrier gas used was helium.

Samples were identified by comparison of elution times and relative positions against standard chromatographs made from combinations of pure compounds. When a question arose as to the identity of a compound, the sample was "spiked"—i.e., reshot with known components added until the presence of overlapping peaks or increase in peak size eliminated any uncertainty.

For the few samples sent to another laboratory, the gas chromatographic conditions and equipment used were as follows:

Gas chromatograph: Perkin Elmer 3920

Columns: 50' stainless-steel support, open tubular columns coated with SP-2100 or OV-17

Carrier gas: helium at 10 psi

Initial temperature:  $30^{\circ}\text{C}$

Program rate:  $8^{\circ}\text{C}/\text{minute}$

Final temperature:  $200^{\circ}\text{C}$  with infinite hold

Injector temperature:  $250^{\circ}\text{C}$

Detector temperature:  $250^{\circ}\text{C}$

Data acquisition with a Spectro-Physics AutoLab System 1 or a Perkin Elmer Sigma 10

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